Initial Evaluation of the Status of Hermes copper (*Lycaena hermes*)

On Conserved Lands in San Diego County

Prepared For:

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Introduction

The Hermes copper butterfly, *Lycaena* [*Hermelycaena*] *hermes* is a rare butterfly endemic to the coastal sage scrub (CSS) community in San Diego County and northern Baja California. Conservation groups and wildlife agencies recognize that Hermes copper is threatened by recent urbanization and wildfires; however this species has yet to be listed as threatened or endangered under the Endangered Species Act. Until recently, relatively little was known about Hermes copper and this lack of information resulted in negative 90-day findings by Fish and Wildlife Service (USFWS) to list the species in 1993 and 2006 (US Fish and Wildlife Service 1993, 2006). However, in 2010 a positive 90-day finding was issued, and the species is currently being reviewed for listing. This project provides an initial evaluation of Hermes copper populations on conserved land in San Diego County from the 2010 flight season.

Previous Hermes Copper Research

Since its initial description in 1870 (Edwards 1870), little information has been collected about the biology and ecology of the Hermes copper butterfly (Figure 1, inset). Seventy years after its initial description only one paper discussing the life history of the butterfly appeared in the literature. All other interest on the butterfly had been strictly from a taxonomical perspective. In 1963 Thorne published the first comprehensive paper on the Hermes copper butterfly, which included information on life history, ecology and behavior.

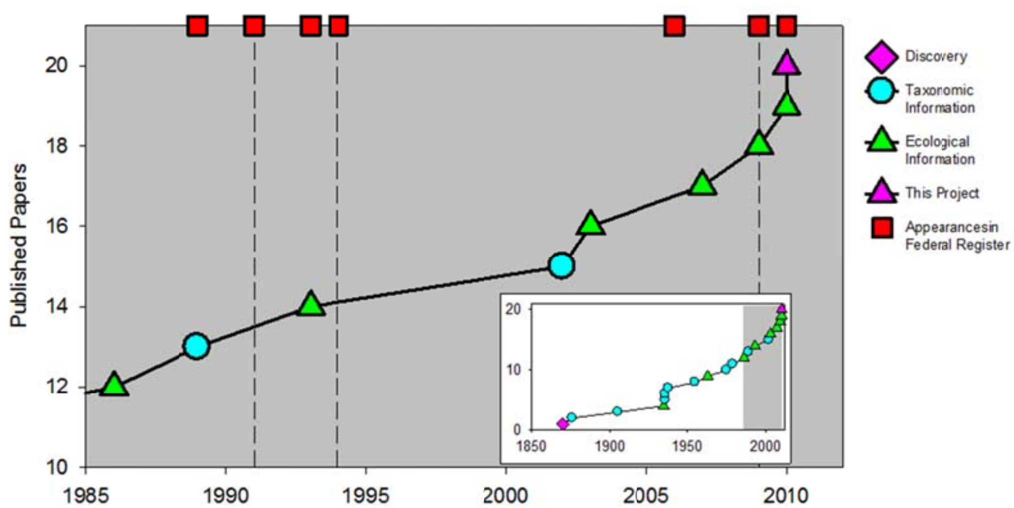


Figure 1: Publications mentioning Hermes copper butterfly. Red squares mark appearances in the Federal Register, including petitions for listing and 90-day findings. Blue circles show papers published with information relevant to the taxonomy of Hermes copper. Green triangles represent papers published with information relevant to Hermes copper ecology. The dashed lines represent regulatory actions regarding the species (e.g. petitions to list and other regulatory activities). The inset figure represents the period from the first description of Hermes copper to the current project.

By 1986 the body of work on Hermes copper consisted of only three papers with information on the ecology of Hermes copper, and nine papers concerning the taxonomy of the species (including the initial description; See Figure 1). Although works in peer-reviewed journals do not always include everything known about a species (indeed local specialists often provide an untapped reservoir of information that is never published), it is a good indicator of relative interest in the species. In 1989, USFWS issued a notice of review, on which Hermes copper was listed as a category 2 species:

> *"Category 2 comprises taxa for which information now in possession of the Service indicates that proposing to list as endangered or threatened is possibly appropriate, but for which conclusive data on biological vulnerability and threat are not currently available to support proposed rules."* (US Fish and Wildlife Service 1989)

Although the Service discontinued the Category 2 status (US Fish and Wildlife Service 1996), this illustrates the earlier concerns about status of the species and general lack of Hermes copper biological knowledge. In 1991 the first petition to list Hermes copper was received, however no additional information appeared in the peer reviewed literature. This petition was met with a negative 90-day finding, stating that there was not enough information of "biological vulnerability" to support listing the species. This petition was updated in 1994 after the appearance of another paper in the literature, but was again met with a negative 90-day finding for the same reason.

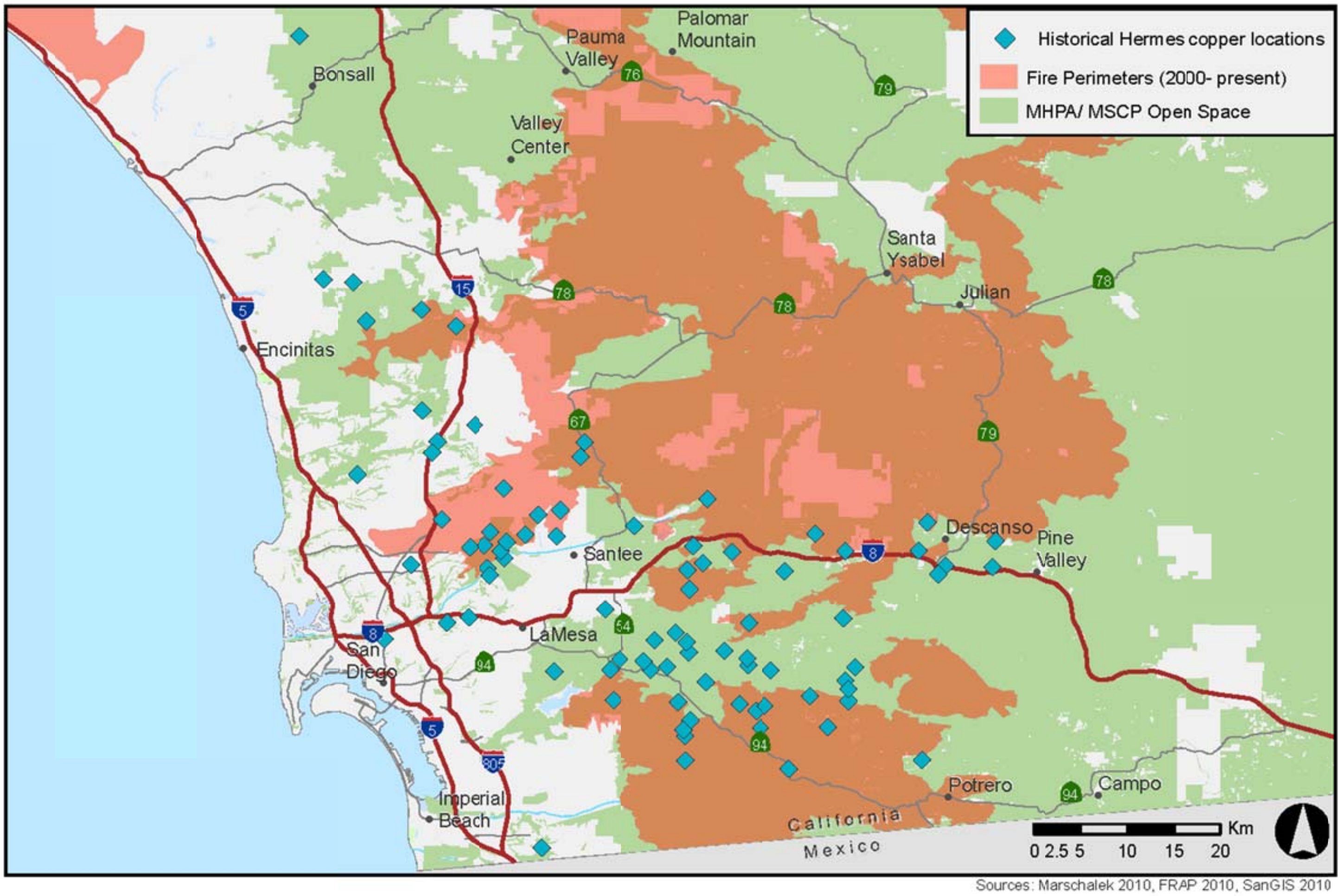
Almost a decade later, Daniel Marschalek, a California Department of Fish and Game (CDFG) employee, finished a Master's thesis regarding Hermes copper (Marschalek 2004). This work marked a turning point in the body of knowledge available on Hermes copper. Additional research on the ecology of Hermes copper was published by Marschalek and his co-authors in 2008, 2009 and 2010 (Marschalek and Deutschman 2008; Marschalek and Deutschman 2009; Marschalek and Klein 2010).

In 2009, a complaint was filed regarding the initial response to the 2004 petition to list the species. This complaint resulted in a positive 90-day finding. At the time of this writing Fish and Wildlife Service is in the 12-month review period pursuant to the positive 90-day finding.

Hermes Copper Biology

In the United States, Hermes copper is only found within San Diego County, west of the Cuyamaca Mountains (Thorne 1963; Brown 1991; Faulkner and Klein 2004; Marschalek 2004; see Map 1). They also occur in northern Baja California, Mexico, however very little is known about the status of the butterfly south of the United States-Mexico border (Thorne 1963; Emmel and Emmel 1973; Marschalek and Klein 2010). They have been recorded as far north as the community of Fallbrook, in San Diego County and as far south as Ensenada in Mexico. They have never been recorded along the Pacific coast, and have not been found further east than the western slopes of the mountains above 1300 meters (Marschalek and Klein 2010).

Hermes' emerge in the late spring after overwintering as eggs and spend a short period of time as caterpillars (Thorne 1963; Faulkner and Klein 2004). Adult emergence is fairly consistent, generally beginning in mid- to late May, with the flight period extending to between late June and mid-July (Faulkner and Klein 2004; Marschalek and Deutschman 2008; Marschalek and Klein 2010). Emergence appears to be influenced by climactic conditions; however our understanding of this relationship is incomplete. For example, 2010 was cool and moist and the Hermes flight season was delayed. In contrast, 2006 was hot and dry and also had a late emergence period (Marschalek and Klein 2010). More comprehensive data are needed to understand this relationship. Virtually nothing conclusive is known about the ability of eggs and larvae to undergo diapause during years with poor conditions.



Map 1: Historical range of Hermes copper. Adapted from Marschalek and Klein 2010.

Hermes larvae use only spiny redberry (*Rhamnus crocea*) as a host plant (Thorne 1963; Brown 1991; Faulkner and Klein 2004). Eggs are laid, typically, at the intersection of branches on new growth (Marschalek and Deutschman 2009). Although adults nectar almost exclusively on California buckwheat (*Eriogonum fasciculatum*) they are rarely found far from a spiny redberry plant (Thorne 1963; Brown 1991; Faulkner and Klein 2004; Marschalek 2004). A more detailed understanding of suitable habitat is lacking. For example, it is not clear how much redberry and/or buckwheat is necessary to support a Hermes copper population in a given area.

During the flight season, Hermes copper adults become active at around 22°C (72°F) (Marschalek 2004; Marschalek and Deutschman 2008). Adult males have a strong preference for openings in the vegetation, including roads and trails, specifically for the north and west sides of openings (Marschalek 2004; Marschalek and Deutschman 2008). Likewise they prefer to perch on the south and east sides of shrubs (Marschalek 2004; Marschalek and Deutschman 2008). They tend to remain inactive or sluggish under conditions of heavy cloud cover and cooler weather (Marschalek 2004; Marschalek and Deutschman 2008).

Hermes copper typically exhibit short movements even under optimal conditions. The majority of their movements tend to be well under 50 meters (Marschalek 2004; Marschalek and Klein 2010). Movements only rarely exceed 100 meters, and the longest movement reported for a Hermes copper is just over 1 kilometer (Marschalek 2004; Marschalek and Klein 2010).

Goals and Objectives

This project was conducted to address growing concerns about the status of Hermes copper. This project was organized around five individual tasks, each a critical part of understanding the status of Hermes copper in San Diego.

Table 1: Project goals and objectives given task by task.

Task A: GIS Analysis
Identify known redberry locations
Identify areas with a high probability of having redberry
Task B: Landscape Genetics
Evaluate dispersal ability
Evaluate post-fire recolonization rates
Process specimens taken in previous years using AFLP
Task C: Vegetation Survey
Field surveys to assess reported patches of redberry
Field surveys to identify unrecorded patches of redberry
Task D: Hermes Copper Field Surveys
Survey as many viable habitat patches as possible
Revisit sites established by Marschalek in previous years
Task E: Data Analysis
Synthesize and analyze this year's data
Report on the current range of Hermes copper in San Diego
Study population structure, habitat suitability and survey methods

The primary goal for tasks A, C and D was to search for previously unreported habitat patches occupied by Hermes copper on conserved lands throughout San Diego County and to establish occupancy. First, potential habitat was identified using existing information on the distribution of the host plant, spiny redberry **(Task A)**. Potential suitable habitat was surveyed before the Hermes copper flight season to further prioritize sites for monitoring and to establish monitoring routes **(Task C)**. During the flight season, standardized routes were visited several times and surveyed for Hermes copper adults **(Task D)**. As part of Task D, we also surveyed routes established by Marschalek starting in 2003. Several of these sites burned in the 2003 and/or 2007 wildfires. Revisiting these sites allowed us to check for re-colonization events and evaluate inter-annual fluctuations in population size.

Although some of the historical sites we visited were in areas burned in 2003 and/or 2007, we did not have time to check all previously identified populations of Hermes or redberry inside the fire perimeters. Although evaluating if populations survived the fires is a critically important question, it was not our primary focus. Data collected after the fires suggests that re-colonization is extremely rare, even when adequate redberry is present (Marschalek and Klein 2010). Since our primary focus was to identify new populations, we decided to expend the majority of our time and effort at the sites with the highest probability of occurrences in 2010. Other entities, such as US Fish and Wildlife personnel surveyed areas that burned since 2003, so the need to revisit burned populations was not entirely unmet this year.

Task B represented a very different approach to understanding the status of Hermes copper. We analyzed genetic material collected during previous field seasons. We used amplified fragment length polymorphism (AFLP) to characterize the genetic differences among individuals both within and among different sites. We hoped that this data on genetic differences would allow us to draw inferences about dispersal events, re-colonization, and population structure.

Task E was the compilation of all that we accomplished during this project. The results are presented in this final report. This report is organized around the major tasks of the project. For each task, we present information on our methods, summarize the results, and discuss their relevance. For simplicity, tasks A and C are combined into a single section.

Tasks A and C – *Identifying Potential Habitat*

The Hermes copper flight season is short — typically lasting between four and six weeks. One of our first tasks was to identify and prioritize potential habitat. We identified potential habitat in two phases. First we used existing information to select and prioritize places likely to contain suitable habitat. Suitable habitat was defined as CSS containing at least some spiny redberry. Second we conducted field reconnaissance to confirm the presence of spiny redberry at those locations.

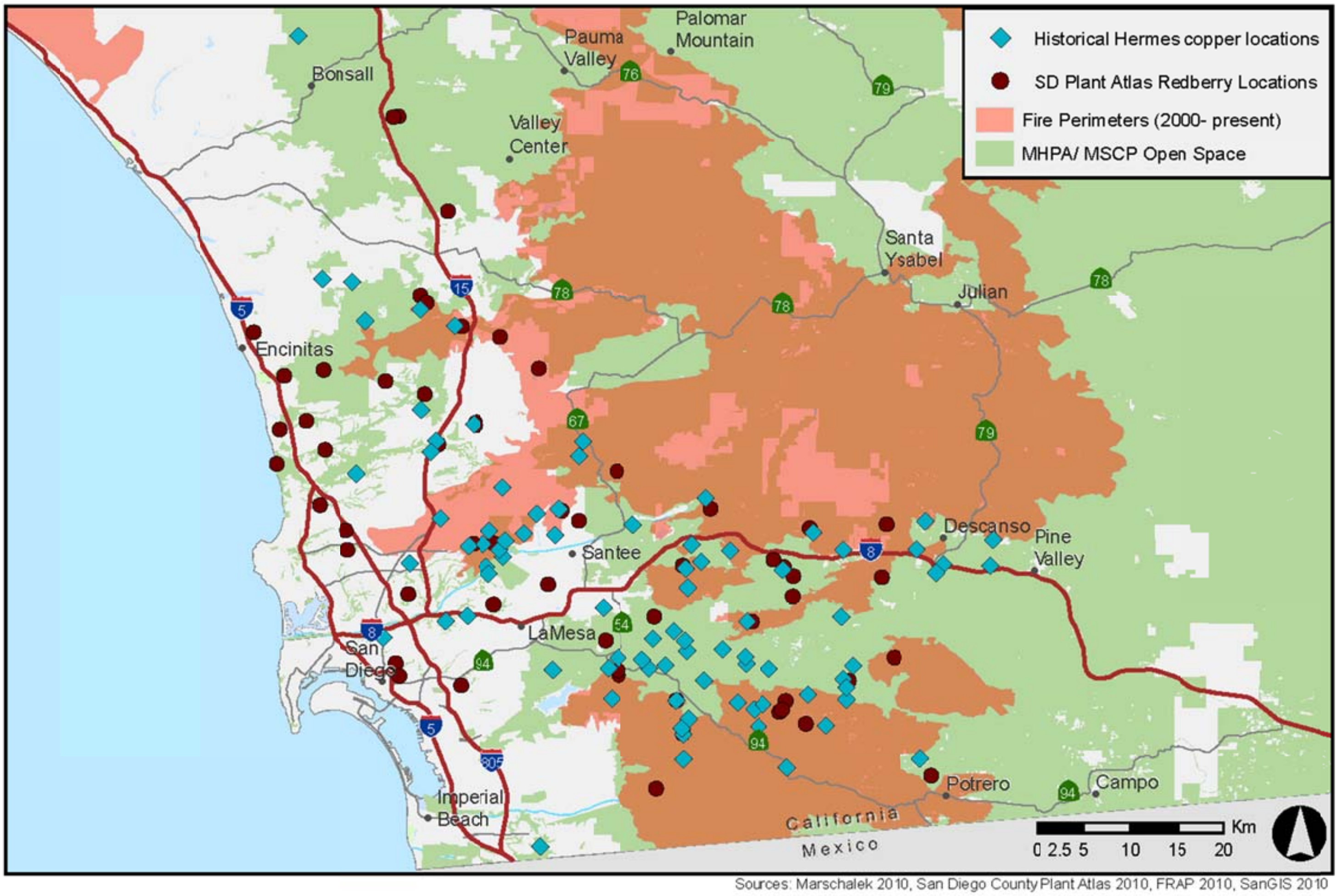
We used four sources of information to identify and prioritize areas that could have potential habitat.

1. **Historical Hermes locations:**
   Areas in and around historical records of Hermes copper were considered likely to contain potential habitat (Map 1).

2. **Informal reports of Hermes presence and suggestions from experts:**
   Numerous informal accounts of Hermes copper exist that are not, to our knowledge, formally confirmed. These accounts often do not have specific location information, so reconnaissance was necessary to identify the specific locations with Hermes copper. In addition multiple individuals within the county and other organizations with "boots on the ground" were solicited for information about potential habitat and/ or butterfly sightings, and these areas were also checked.

3. **Spiny redberry locations as reported in the Plant Atlas**
   Locations given in the Plant Atlas are not all-inclusive, and not precise; however this data gave us a good understanding of where in the county spiny redberry was most likely to be found, and where it was the most concentrated (Map 2).

4. **2003 and 2007 fire maps**
   To our knowledge Hermes copper rarely survive fire as their eggs burn along with the host plant. In addition, re-colonization is slow, particularly after events that destroy large areas of habitat. As a result, areas that have burned since the 2003 Hermes copper flight season were a lower priority for this project than areas that went unburned because re-colonization is unlikely (Map 2).

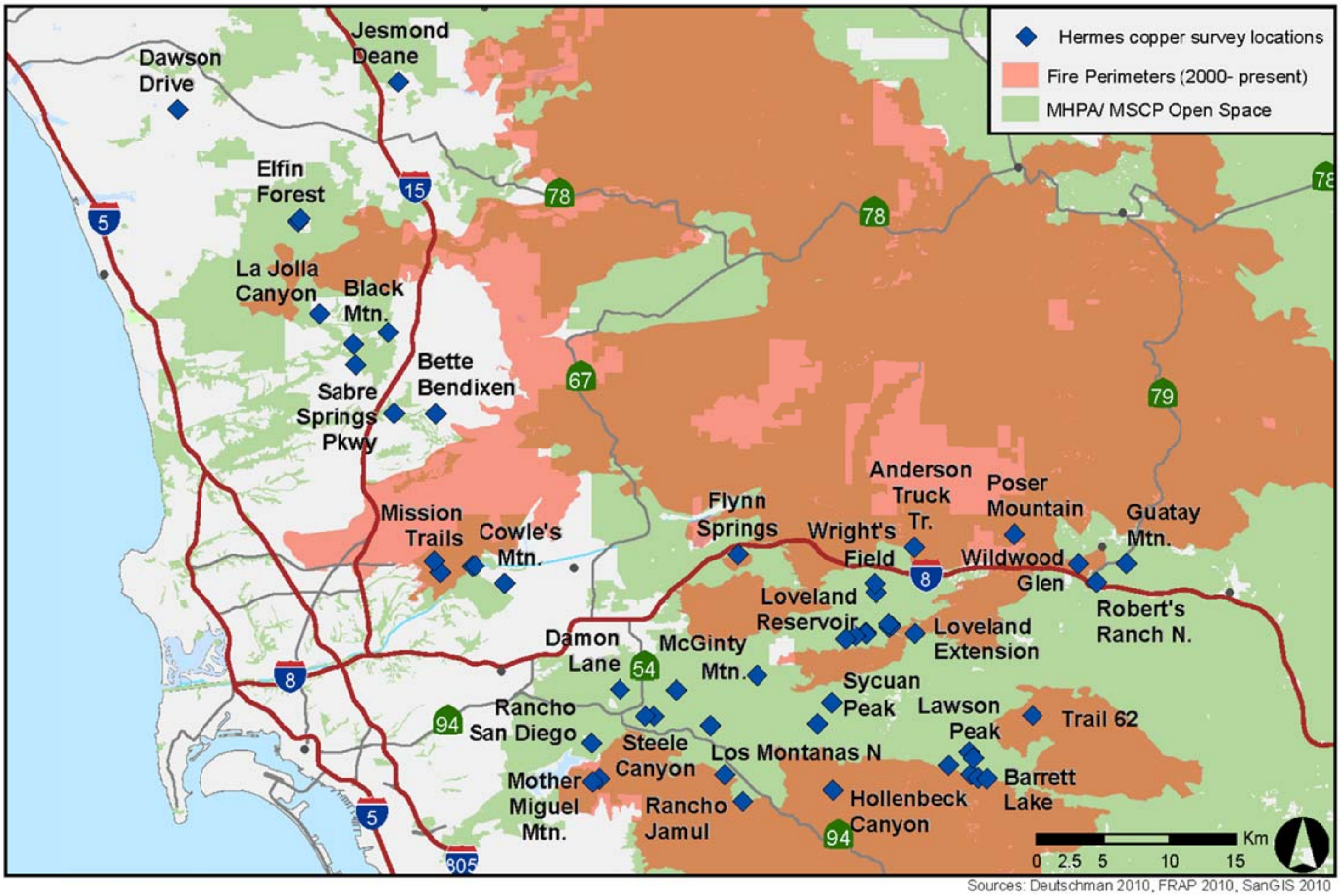


Map 2: Information used to determine redberry reconnaissance. This included historical Hermes copper records, plant atlas redberry data, and major fire perimeters.

By overlaying these four sources of information we identified areas that were most likely to be inhabited by Hermes copper, and were able to prioritize them for reconnaissance searches (Map 2). This exercise yielded a total of 66 priority areas which we searched for redberry (See Appendix 1). These areas did not include locations which recently burned unless there was a question of fire perimeter, nor do they include open space in highly urbanized areas unless larger patches of open space with potential redberry or recent Hermes sightings were nearby.

Of those 66 areas 42 had at least some redberry plants, and 36 had more than a few scattered individuals (See Appendix 1). We prioritized areas based on the relative density of the redberry at the site, the degree to which the surrounding area was urbanized, and current land use. Based on this information we defined sites (areas of contiguous open space) and routes (survey transects within a site).

We arrived at a total of 33 sites and 56 routes (collaborating with Alison Anderson and John Martin at USFWS these numbers increased to 35 and 61) that were to be checked for Hermes copper during the flight season (Map 3, Appendix 2). Some of Martin's sites and routes were not on the list of highest priority because those habitats burned, but are included to increase the survey efforts, particularly in regards to documenting recolonization events.



Map 3: 2010 Deutschman lab Hermes copper survey locations. Blue diamonds represent survey routes. Labels reflect site names as used by many local scientists and land managers. Note some site labels are suppressed because of spatial overlap. The full list is contained in Table 8.

Task B – *Landscape Genetics*

Goals and Objectives:

For Hermes copper, mark and recapture methods are often inadequate for detecting long distance movements. Widely varying temporal and spatial scales, typically low recapture rates, and an inability to determine if an individual has been recruited into the breeding population (even in cases of successful recaptures) create substantial obstacles for such methods. Estimates of genetic variability, combined with inferences of the genetic population structure, provides a means to evaluate the magnitude of differentiation within and among these populations, all of which indicate dispersal ability (gene flow). Increased genetic differentiation suggests that populations are isolated from each other, perhaps even leading to local adaptation. Integrating the genetic data with natural history and landscape features will suggest factors important for the persistence of the species and development of conservation practices. If populations are found to be completely isolated genetically, this would pose radically different policy considerations to conservation efforts than if the populations were all similar.

We used 145 AFLP markers to estimate fundamental population genetic parameters including (1) polymorphism, (2) expected heterozygosity, (3) F_{ST} values, and (4) private alleles to provide insight into the population structure of Hermes copper. We used these parameters to evaluate the magnitude of differentiation within and among these populations which indicates dispersal ability (gene flow). Integrating the genetic data with the natural history and landscape features suggests several factors important for the short and long-term conservation of the species.

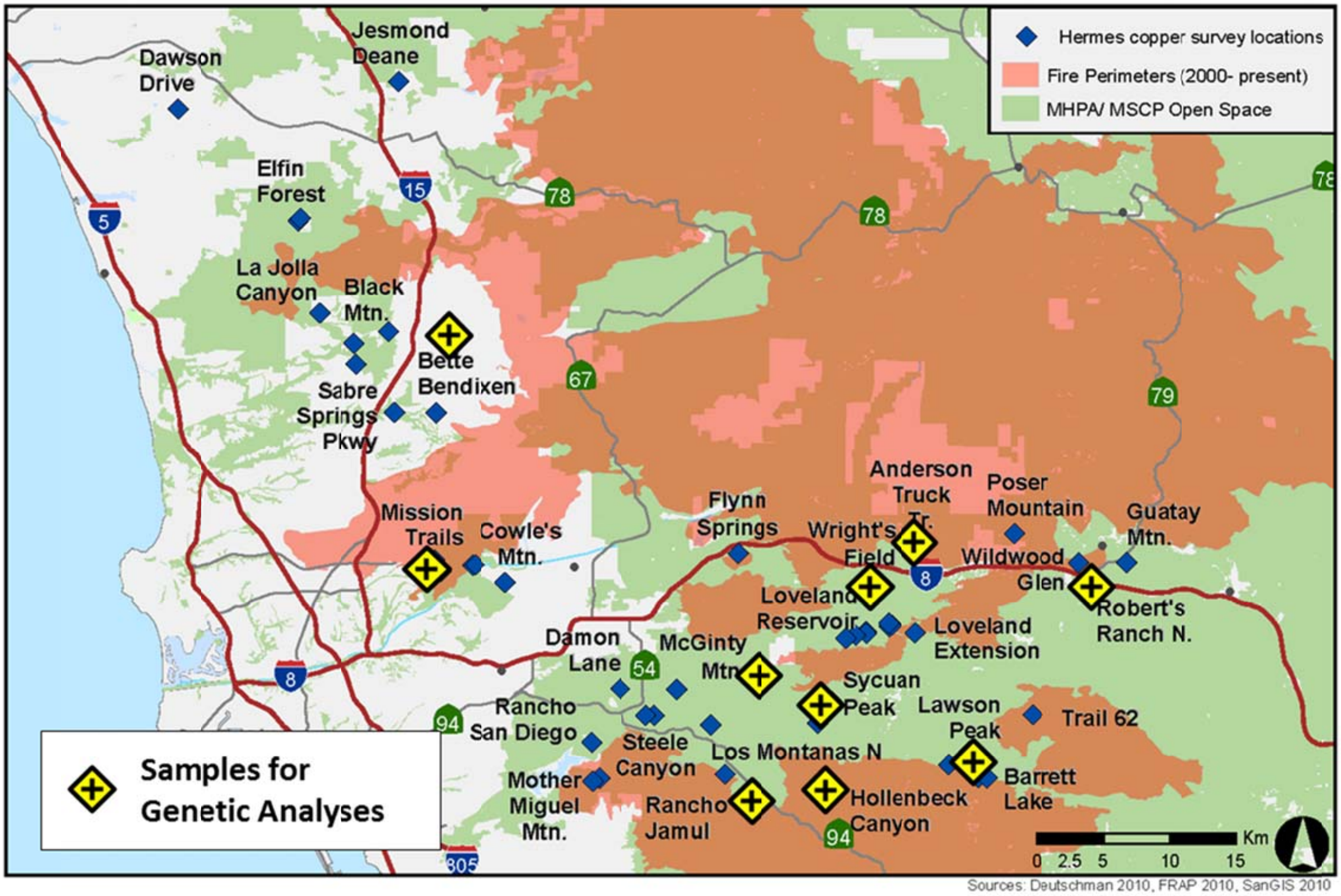
Methods:

Field Work

We obtained a total of 86 specimens from 15 locations in four collecting efforts (Map 4, Table 2), with one location (Wildwood Glen / Descanso) sampled in three different years and another location (Lawson Valley) sampled two different years. Of these, five were extirpated in the 2003 or 2007 wildfires, 10 were unaffected by fires in at least the last decade, and one was sampled after recolonization from the 2003 fires. These locations represent the north-south and east-west extremes of Hermes copper range and nearly all known populations in the United States within the last decade.

Molecular Procedures

Many genetic marker systems exist, but the selection of a specific technique must at least insure an ability to detect genetic variation at the level of individuals for a population-based study. Amplified fragment length polymorphism (AFLP) method meets this requirement by sampling genetic differences at an individual level (Vos *et al*. 1995). AFLP can be applied to genetically non-characterized species and requires only a short time to implement, benefiting conservation efforts. The AFLP technique provides information which is used to estimate basic population genetic parameters including the magnitude of polymorphism and heterozygosity (Berres et al. *in review*). We also applied well-understood population genetic models to evaluate the genetic structure of Hermes copper (differentiation among individuals within and between populations) and evidence of dispersal ability. We used the trace analysis program DAx 8.0 to visualize the allelic data; AFLP-SURV (Vekemans 2002, Vekemans et al. 2002) to calculate polymorphism, expected heterozygosity rates, and F_{ST} values; IBDWS (Jensen et al. 2005) to investigate isolation-by-distance (IBD); and GDA (Lewis and Zaykin 2001) to identify private alleles (Table 3).



Map 4: Locations of Hermes copper specimens obtained for genetic analysis. Complete description of individuals sampled can be found in Table 2.

Polymorphic loci are AFLP markers that are found in 5-95% of individuals. If the marker is present in less than 5% or greater than 95% of individuals the marker is considered to be fixed, meaning there is no genetic variation at that particular locus. The percent of polymorphic loci represents the proportion of observed loci which are polymorphic in a group of individuals. Heterozygosity refers to the condition of an individual having more than one allele for a particular gene. Observed heterozygosity rates could not be calculated directly because AFLP cannot distinguish heterozygous loci (i.e. it can only determine presence/absence of a marker peak). Statistical estimators (Lynch & Milligan 1994 and Zhivotovsky 1999) can estimate expected heterozygosity, as reported here. Generally, observed rates of heterozygosity are about 10-20% lower than expected rates indicating a departure from Hardy-Weinberg expectations (e.g. drift, migration, population structure). The reported heterozygosity rates are the proportion of individuals heterozygous at a locus and averaged over all loci. Both polymorphism and heterozygosity are measures of genetic variation and it is believed that populations or species will be better able to adapt to environmental changes with higher levels of variation.

Table 2: Details of Hermes copper specimens obtained for genetic analysis. The table includes year of sampling, location, and the status of the population at each sampling location. Note that a few sites are sampled in more than one year.

Year	Sampling Location	Sample Size	Population Status
2003	Anderson Road	5	Extirpated (wildfire)
	Crestridge ER	4	Extirpated (wildfire)
	Wildwood Glen (Descanso)	5	Extirpated (wildfire) *
	Meadowbrook ER	10	Extant **
	Rancho Jamul ER	10	Extirpated (wildfire)
2006	Hollenbeck Canyon WA	13	Extirpated (wildfire)
2008	Wildwood Glen (Descanso)	3	Recolonization (wildfire) *
	Lawson Valley	5	Extant
	McGinty Mountain ER	2	Extant
	McGinty Mountain TNC	2	Extant
	Mission Trails	5	Extant
	Robert's Ranch	6	Extant
	Wright's Field N	3	Extant
	Wright's Field S	3	Extant
2009	Wildwood Glen (Descanso)	1	Recolonization (wildfire) *
	Lawson Peak	3	Extant
	Lawson Valley	5	Extant
	Sycuan Peak	1	Extant

* Observations of Hermes copper adults associated with recolonization at the Wildwood Glen/Descanso location was first documented in 2007 (Marschalek and Klein 2010).

** Habitat remains relatively undisturbed since last sighting, but surveys during recent years have no Hermes copper observations.

Table 3: Programs used in molecular data analysis.

Program	Task
DAx 8.0	Visualize AFLP Data
AFLP-SURV	Calculate Polymorphism Rate Calculate Expected Heterozygosity Rate Calculate F_{ST} Values
IBDWS	Test For Isolation-By-Distance
GDA	Identify Private Alleles

F_{ST} values are a coarse measure of differentiation by comparing genetic variation within and between populations, which provides indirect evidence of movement between populations (sampling locations). A value of zero indicates that individuals from the sampling locations interbreed (completely panmictic population), while a value of one represents completely isolated populations with no gene flow. Only when there are strong tendencies for long-distance dispersal will F_{ST} values truly be negative; however, calculations may result in negative values when the true F_{ST} equals zero. Since most Hermes copper individuals do not appear to exhibit long-distance dispersal behaviors, negative F_{ST} values should be considered equal to zero (no genetic differentiation).Testing for isolation by distance (IBD) uses F_{ST} values calculated for all possible pairwise comparisons of sampling locations and comparing it to pairwise geographical distances. If the genetic distances do not adhere to IBD, it is likely that a factor other than distance is affecting dispersal (e.g. dispersal barriers, behavior, fire history).

Private alleles represent AFLP markers that are only found in a single sampling location. Because we are treating each year of sampling separately (rather than pooling all data), private alleles are determined for each year and can be present at multiple locations in multiple years. Private alleles also provide insight into the level of genetic differentiation among populations.

Results:

Molecular Procedures

We successfully applied the AFLP process to all 86 Hermes copper specimens. Initial testing provided important information for many steps of the AFLP process. We used the CTAB method for DNA isolation as this technique, compared to conventional kits (e.g. Quiagen), both provided higher yields and purity, removing compounds that have the potential to interfere with subsequent reactions. The first reaction in the AFLP procedure, restriction digestion, we found was reproducible if run overnight, rather than the widely recommended two hour period, and the concentration of digestive enzymes was increased by ten percent compared to the manufacturer’s recommended level. Sixteen primer pairs were screened for the pre-selective step, each assessed with two individuals to evaluate individual variation. Primer screens used EcoRI and either AseI or BfaI, with only one additional pre-selective base (G, A, T, or C) on each of the forward and reverse primers. All but Eco+A/Bfa+C were determined to work adequately to proceed to the selective primer screen. Forty-eight selective primer pairs (pre-selective primer with two additional bases) provided several pairs that produced high-quality fingerprints. We opted to use the primer pair Eco+GT/Ase+TG because of the relatively high number of marker peaks, peaks were more evenly distributed, and the peaks were generally clearly separated so their specific number and location could be determined.

We were able to detect differences between individuals, even those collected within a couple meters of each other (see Figure 2). This variation within a sampling location can be compared to the variation among all sampling locations to provide an estimate of the population structure of Hermes copper. A total of 145 marker loci were included in the analysis. A calculation of F_{ST}, including a confidence interval, requires at least two specimens from each location so single individuals captured in 2009 from Sycuan Peak Ecological Reserve and Wildwood Glen were excluded from this analysis.

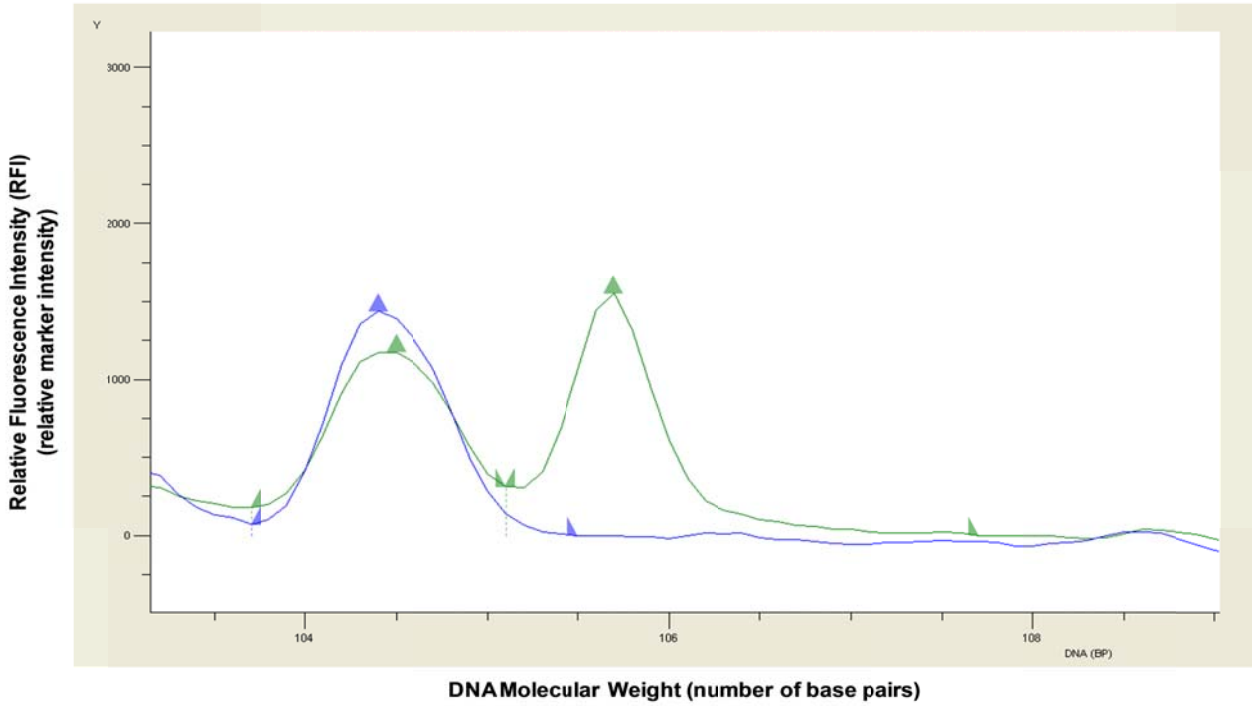


Figure 2: Relative fluorescence intensity (RFI) for two DNA marker fragments for two individuals. These two individuals were collected from Meadowbrook Ecological Reserve about 18 meters apart. Note the shared AFLP marker at 104.5 base pairs and the unique marker for individual 1 (green) at 105.8 base pairs. The genetic interpretation is that the two individuals are monomorphic at 104.5 and polymorphic at 105.8.

Genetic Variability

The percent of polymorphic loci ranged from 53.8% at Wright's Field N to 69.7% at Meadowbrook ER (Table 4). The expected heterozygosity under Hardy-Weinberg equilibrium for each site ranges from 0.127-0.207, with a yearly mean expected heterozygosity ranging from 0.160-0.200. Two locations were sampled during two years each, both showing some change in either proportion of polymorphic loci or expected heterozygosity rates. Individuals sampled at Lawson Valley in 2008 have 62.8% polymorphic loci and 0.171 expected heterozygosity, but 56.6% and 0.165 in 2009. Sampled individuals from Wildwood Glen in 2003 and 2008 have 57.9% and 60.7% polymorphic loci, respectively, but differ almost two-fold in expected heterozygosity (0.127 and 0.207 for 2003 and 2008, respectively).

Table 4: Genetic parameters calculated for each sampled location.

Sample Location	Sample Size	# Polymorphic Loci (145 total)	Percent of Polymophic Loci	Expected Heterozygosity
2003				
Anderson Road	5	88	60.7	0.164
Crestridge ER	4	85	58.6	0.176
Wildwood Glen (Descanso)	5	84	57.9	0.127
Meadowbrook ER	10	100	69.0	0.171
Rancho Jamul ER	10	101	69.7	0.176
		Average:	**63.2**	**0.163**
2006				
Hollenbeck Canyon WA	13	95	65.5	0.200
2008				
Wildwood Glen (Descanso)	3	88	60.7	0.207
Lawson Valley	5	91	62.8	0.171
McGinty Mtn ER	2	79	54.5	0.151
McGinty Mtn TNC	2	65	44.8	0.130
Mission Trails Reg. Pk.	5	88	60.7	0.142
Robert's Ranch	6	87	60.0	0.143
Wright's Field N	3	78	53.8	0.140
Wright's Field S	3	81	55.9	0.196
		Average:	**56.7**	**0.160**
2009				
Lawson Peak	3	78	53.8	0.201
Lawson Valley	5	82	56.6	0.165
		Average:	**55.2**	**0.183**

Genetic Differentiation

Specimens collected for each year were analyzed separately for the purpose of calculating F_{ST} values to avoid a bias toward less differentiation. This reduced differentiation results from lumping different allele frequencies based on temporal, rather than intended spatial sampling, reducing the ability to detect migration. To test for statistically significant population structure, a 95% confidence interval was constructed by permuting individuals across populations. Here, H_o: $F_{ST} = 0$ is tested. If the point estimate of F_{ST} is greater than the upper bound of the 95% CI, there is evidence of genetic population differentiation. Two locations exhibit significantly different allele frequencies if the calculated F_{ST} is greater than the upper limit of the interval.

We evaluated genetic differentiation based on samples in 2003 (n=34 individuals) and 2008 (n=29 individuals). F_{ST} values of 0.0716 (2003) and 0.0988 (2008) indicated significant genetic differentiation relative to a random assemblage of individuals. Both of these values exceeded the 95% confidence regions under the null model and are statistically significant. The pattern of differentiation among populations was complex. In 2003, four of the five largest observed differences were between Meadowbrook ER and the other four locations. In 2008, A pairwise comparison of F_{ST} values showed that several sampled locations were well differentiated from each other while others were nearly identical. The two locations exhibiting the greatest difference are two locations on opposite sides of McGinty Mountain (F_{ST} = 0.3456) suggesting the mountain represents an effective dispersal barrier. The two locations at Wright's Field (F_{ST} = 0.0000) and these two locations compared to Lawson Valley (F_{ST} = 0.0065 and 0.0000 for Wright's Field N and S, respectively) are very similar.

We evaluated whether genetic differentiation was related to geographic distance (e.g. Isolation By Distance, or IBD) in both 2003 and 2008. For 2003, no statistically significant isolation by distance pattern was found (5 populations; Z = 0.9846, r = 0.3291, p = 0.2244). However, the data (Figure 3) is suggestive of IBD. It is possible that the small sample sizes reduced the power needed to detect significance for all but the most extreme cases. Using a Mantel Test to analyze the eight populations from 2008, there is no evidence of isolation by distance pattern (8 populations: Z = 2.9990, r = -0.0448, p = 0.5884) under a two-dimensional stepping stone model.

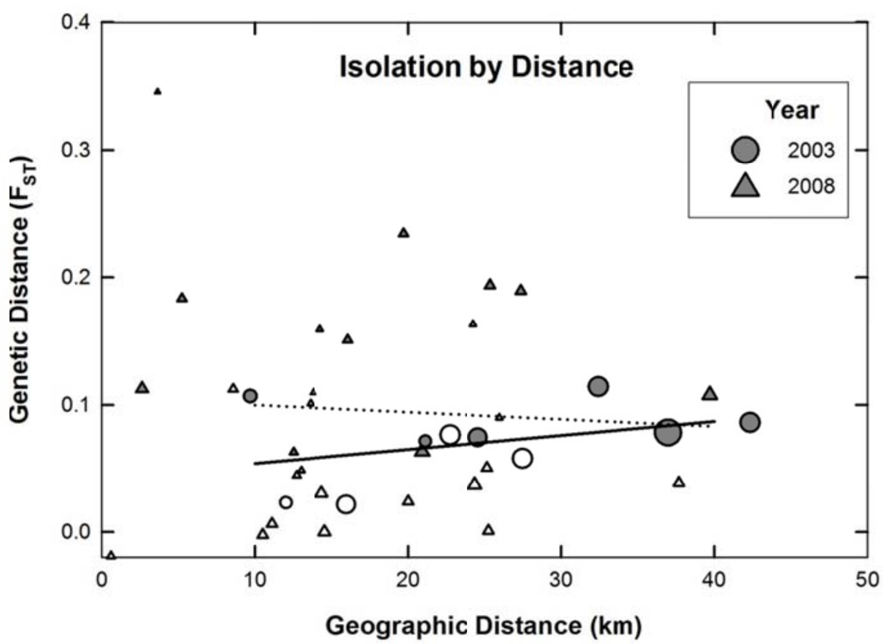


Figure 3: Genetic differentiation and geographic separation. F_{ST} values are plotted for the five populations sampled in 2003 (circles) and the eight populations sampled in 2008 (triangles). F_{ST} values that are significant (outside the 95% confidence envelope) are filled with gray. The relationship between F_{ST} and geographic separation is plotted for 2003 (solid line) and 2008 (dotted line).

Private Alleles

Private alleles are defined as AFLP markers that are unique to a sampling location in a given year. Using private alleles, we are able to obtain evidence of differentiation and movement at the level of individual butterflies. A total of 33 private alleles are present from the 2003 sampling locations and 26 from the 2008 sampling locations. Five private alleles are common between 2003 and 2008 suggesting migration between these sampling locations, with distances between the sites sharing private alleles ranging from 5.3 to 38.5 km (Table 5a). Individuals sampled at HCWA in 2006 have alleles matching six private alleles from seven locations, as one allele was found from HCWA, Meadowbrook ER, and Wright's Field S (Table 5b).

Table 5: Private alleles shared between: a) 2003 and 2008 sampling locations, and b) Hollenbeck Canyon Wildlife Area and sampling locations of 2003 and 2008.

a. Shared Private Alleles of 2003 and 2008 Locations

Locus	2003 Location	2008 Location	Distance Between Locations (km)
80	Crestridge ER	Wildwood Glen (Descanso)	21.1
107	Meadowbrook ER	Mission Trails	14.3
124	Crestridge ER	Wildwood Glen (Descanso)	21.1
127	Wildwood Glen (Descanso)	Mission Trails	37.7
145	Meadowbrook ER	Wright's Field S	31.7

b. Private Alleles Shared with Hollenbeck Canyon Wildlife Area

Locus	Location	Year	Distance From HCWA (km)
1	McGinty Mtn ER	2008	10.9
2	Crestridge ER	2003	15.5
25	Mission Trails	2008	26.9
43	Wildwood Glen (Descanso)	2003	22.8
87	Rancho Jamul ER	2003	5.3
145	Meadowbrook ER	2003	38.5
145	Wright's Field S	2008	14.7

Discussion

Using AFLP we detected differences between individuals and at very fine spatial scales ranging from 0.5 to 42.3 km. Our analysis was able to detect genetic structure within the San Diego County distribution of Hermes copper. This genetic structure varied over spatial and temporal scales and supports the presence of multiple populations of Hermes copper. Despite this genetic structure, evidence of distance dispersal and subsequent recruitment into the breeding population does exist.

F_{ST} analysis indicated complicated patterns of genetic differentiation across the landscape. Although not statistically significant (a function of small sample size), isolation-by-distance appears likely for those individuals present at the edges of the distribution within San Diego County. When comparing many locations well within the distributional extent, other factors such as higher rates of migration or specific dispersal barriers may be more influential in determining structure. In both 2003 and 2008, the sampling location most geographically isolated (Meadowbrook ER and Mission Trails, respectively) was genetically distinct from most of the other sites. There are also several examples with sampling locations in close geographical proximity but exhibit high levels of genetic differentiation. The most proximal locations in 2003 (Anderson Road and Descanso) provides the second highest level of differentiation while the closest pair in 2008 (the two locations from McGinty Mountain) has the greatest differentiation, likely due to limited dispersal between the areas. The two McGinty Mountain locations are on opposites sides of the mountain, suggesting that the topography acts as a dispersal barrier.

Although the 2003 overall F_{ST} is lower than that of 2008, the sampling locations of 2003 are more genetically differentiated. A likely explanation is the spatial arrangement of the sampling locations as the 2003 samples represent distributional extremes and suggest isolation by distance theory, while the 2008 samples are more restricted geographically, with the exception of Mission Trails Regional Park. A comparison of pairwise distances between sampling locations demonstrates that the 2003 locations (mean = 24.5 km, st. dev. = 10.6 km) are more separated than the 2008 locations (mean = 17.3 km, st. dev. = 9.7 km).

Based on levels of genetic differentiation and tracking private alleles, there appears to be greater connectivity of the eastern sampling locations compared to the others. Locations such as Lawson Valley, Lawson Peak, and Wildwood Glen are in an area with a relatively higher density of recent Hermes copper observations. In addition, this area of San Diego County is relatively undeveloped and likely contains a greater degree of continuous coastal sage scrub habitat. Much of this habitat is protected by Cleveland National Forest, San Diego National Wildlife Refuge, and McGinty Mountain and Sycuan Peak Ecological Reserves.

A finer-scale perspective also supports regular movement of individuals between areas less than 1.0 km apart. Analysis of specimens from HCWA indicated migration between all three sampled locations because of the lack of differentiation (F_{ST}= 0.0000). Adult movement between two of these locations separated by a riparian oak woodland was not detected by traditional marking studies of moderate effort (Marschalek and Klein 2010). A lack of genetic differentiation at a similar scale was observed at Wright's Field in 2008, with two sampling locations separated by a grassland of about 600 meters wide.

Recolonization of habitat following wildfires critical to the survival of the species, yet has rarely been documented (Marschalek and Klein 2010). Wildwood Glen is the one location we observed recolonization and a comparison of individuals before and after the fire indicated genetically differentiated groups. The post-fire individuals also were significantly different from those of Robert's Ranch, known occupied habitat 2600 m away. This also supports the idea that Hermes copper individuals are capable of making make long-distance movements but there may be landscape features that either enhance or restrict dispersal. While we cannot absolutely rule out temporal effects as seen at Lawson Valley in the absence of habitat disturbance, it is very unlikely.

While genetic similarity suggests exchange of individuals, genetic differentiation of individuals from two locations could be a result of dispersal barriers, genetic drift, result of original colonizers, or a combination of factors. The frequency of sampling locations that are genetically similar and widely separated geographically would disagree with results from marking studies which observed that most individuals moved less than 200 m (Marschalek and Deutschman 2008, Marschalek and Klein 2010). The majority of these individuals were territorial males, so it is possible that Hermes copper exhibits sex-biased long distance dispersal by females. This is the case with *Lycaena arota* (Scott 1973a), other lycaenids (Robbins and Small 1981), and a review of mark-recapture studies of 11 butterfly species (Scott 1973b). In general, lycaenids are not very vagile (New 1993) and wind may assist movements

(Robbins and Small 1981). An ability to genetically sex these individuals would allow us to evaluate this phenomenon.

Investigation of genetic structure in relation to temporal scales is rare in population genetic studies and appears to be important for understanding population dynamics of Hermes copper. Not only did we find that pre- and post-fire populations differ genetically (Wildwood Glen), but our data suggests that the genetic composition of a population may change over a relatively short time period (Lawson Valley). At this time it would be difficult to determine if the temporal variation of allele frequencies is due to genetic drift and/or dispersal. The relatively small population sizes of Hermes copper are more likely effected by these factors than species with larger population sizes.

Conclusions

The AFLP process was able to detect genetic differences among individuals, even those captured within meters of each other. Analyses support modest levels of polymorphism and heterozygosity. Based on the spatial arrangement of genetic variability, Hermes copper in San Diego County is not one panmictic population because dispersal appears to be restricted in some locations. In contrast to previous reports and mark-release studies, our genetic analysis indicates that individuals may move more freely throughout some portions of the landscape. Our analyses also show that the genetic composition of individuals at any location exhibit a high degree of temporal variability, possibly due to biotic (drift, dispersal) and abiotic (landscape, fire regime) influences. Because our data indicates a strong temporal component, we recommend continued sampling of these locations in future years.

Future Study

Specimen collection for this study was opportunistic with limited resampling due to frequent large scale wildfires in the last decade, the discovery of occupied habitat patches (Marschalek and Klein 2010), and dry weather resulting in lower adult numbers. Our understanding of Hermes copper long-distance movements and habitat patch connectivity would benefit from including additional specimens to the current dataset. These specimens would increase the spatial extent of genetic information and more completely describe the genetic structure of the species after the recent wildfires. Specific topics that warrant further investigation include year-to-year genetic variability, post-fire recolonization characteristics, population assignment tests to detect original populations of dispersing individuals, and potential sex-biased dispersal.

Task D – *Field Surveys*

Preparation

In order to prepare for flight season surveys we developed a training and testing program for the field team. Team members were provided a list of butterfly species detected by Marschalek during butterfly surveys in previous years (Table 6)

Table 6: Common names of butterflies detected during previous studies. List compiled by DA Marschalek.

American Lady	Hedge-Row Hairstreak
Behr's Metalmark	Hermes Copper
Bernardino or Dotted Blue	Lorquini's Admiral
Boisduval's Blue	Lupine or Acmon Blue
Brown Elfin	Marine Blue
Buckeye	Monarch
Cabbage White	Mt. Mahogany Hairstreak
California Dogface	Northern White Skipper
Comstock's Fritillary	Orange Sulphur
California Hairstreak	Painted Lady
California Ringlet	Pale Swallowtail
California Sister	Pygmy Blue
Checkered White	Queen
Cloudless Sulphur	Reakirt's Blue
Dainty Sulphur	Red Admiral
Edward's Blue	Rural Skipper
Fiery Skipper	Sara's Orangetip
Funeral Duskywing	Silver Spotted Skipper
Gabb's Checkerspot	Silvery Blue
Gray Hairstreak	Sleepy Orange
Great Copper	Sylvan Hairstreak
Great Purple Hairstreak	Tiger Swallowtail
Great Basin Wood-Nymph	West Coast Lady
Hartford's Sulphur	White Checkered Skipper

The team then studied images, descriptions and specimens of these species (Figure 4). Team members were allowed to look at the underside and topside of the wings while familiarizing themselves with the species, and had access to two field guides which described distinguishing characteristics. A set of study aids was developed which incorporated images and descriptions of the distinguishing characters for some groups of species (such as blues and hairstreaks) which had several similar looking species. To reduce the time spent identifying species other than Hermes copper in the field, we grouped Bernardino and dotted blue, and acmon and lupine blues because they are very similar and can be quite common in certain locations.

Figure 4: Picture of specimen box arranged for training and testing field crew. Specimens on loan from the San Diego State University collection.

Team members had to pass a test before becoming certified to conduct surveys. The test consisted of three sections: identification of species in images, identification of pinned specimens, and identification of butterflies in digital videos. Partial credit was given for identification to subfamily/ group (e.g. sulfurs, whites, blues, etc.) for the specimen (1 point for group, 3 for species) and video (2 points per group, 3 for species) sections. Misidentifying a Hermes copper resulted in automatic failure of the test (e.g. mistaking something for a Hermes, or mistaking a Hermes for something else).

Table 7: Points assigned for Hermes copper field survey qualification test.

	Questions	Partial Credit	Full Credit	Section Points	Section %
Images:	30	0	1	30	25%
Pinned Specimens:	20	1	3	60	50%
Digital Video:	10	2	3	30	25%
			Total:	**120**	**100%**

In order to qualify for conducting surveys of Hermes copper butterflies, team members had to meet the following qualifications:

- Identify all Hermes copper butterflies correctly (any misidentification was an automatic disqualifier until the next test round)
- Score 75% or greater on the butterfly identification test described above.
- If a test taker fails to qualify they can retest each week with a different batch of images and specimens, as long as they have been in the field with a qualified person doing surveys for practice.
- Observe at least one Hermes copper butterfly in the field with a currently qualified person (Dan Marschalek, Mike Klein) prior to conducting surveys (applies to new surveyors—previously approved surveyors need to see a live Hermes once a year to stay current and be able to pass the test prior to the flight season each year.

The testing rules were as follows:

- Only species that occur concurrently (temporally and spatially) with Hermes Copper will be on the test.
- Specimens and images may appear with either the upper or under side of the wings in view (videos are the same, based on what footage was captured).
- The test will be timed
    - 20 minutes for the image identification section.
    - 20 minutes for the specimen identification section.
    - The length of the video is the limiting factor during the video section. Videos cannot be re-started or paused.
- Field guides and other aids are not allowed.

Hermes copper Surveys

We used the Skyline Truck Trail as an indicator site based on Marschalek's data from previous years. In his observations, this site generally has early emergence of Hermes copper butterflies and is very easy to reach. We began checking the Skyline Truck Trail site the week of May 17th, which we anticipated to be very near the start of the flight season. We made trips to this site regularly until May 29th when we recorded our first observations of Hermes copper. May 29th represents a considerably later start to the season than we anticipated, although it is within the range of emergence periods described in the literature.

On May 29th one Hermes copper was present at Skyline Truck Trail, and two were present on Sycuan Peak. After this date teams began cycling through a total of 35 sites with 61 routes at the shortest interval possible, about once a week for most sites (Table 8). Our effort across sites was not homogenous, based on the priority of the site, the status of the buckwheat at the site, and how long the Hermes copper persisted if they were present. Most top priority sites received three or more visits between May 29th and July 9th. Butterfly densities throughout this time period are given in the next section.

Table 8: Hermes copper survey locations and counts.

Site Name (Observer)	New Site	Number of Routes	Number of Visits	**Total Hc Observed**	Max Hc Observed
McGinty Mountain	**Yes**	3	7	**62**	26
Sycuan Peak	**Yes**	1	9	**45**	12
Skyline Truck Trail		1	15	**37**	9
Loveland Reservoir	**Yes**	4	5	**8**	3
Wrights Field *(Klein)*		1	3	**7**	*NR*
Roberts Ranch North		1	4	**5**	4
Los Montanas North		2	4	**5**	3
Lawson Peak		1	4	**4**	2
California Riding and Hiking Trail	**Yes**	4	4	**3**	2
Los Montanas South		1	4	**3**	1
Wildwood Glen		1	5	**2**	1
Loveland Extension	**Yes**	2	4	**1**	1
Mission Trails		4	4	**1**	1
Anderson Truck Trail		1	2	**0**	0
Barrett Lake		4	3	**0**	0
Bette Bendixen Park		1	3	**0**	0
Black Mountain *(Simonsen-Marchant)*		3	7	**0**	0
Cowels Mountain		1	4	**0**	0
Crestridge		3	4	**0**	0
Damon Lane		1	3	**0**	0
Dawson Drive *(Anderson)*		1	4	**0**	0
Elfin Forest *(Anderson)*		1	3	**0**	0
Flynn Springs *(Klein)*		1	2	**0**	0
Guatay Mountain		2	2	**0**	0
Hollenbeck Canyon		1	2	**0**	0
Jesmond Dene Park *(Anderson)*		1	3	**0**	0
La Jolla Canyon		1	2	**0**	0
Marron Valley		1	1	**0**	0
Meadowbrook		2	3	**0**	0
Mother Miguel Mountain *(Martin)*		2	3	**0**	0
Rancho Jamul		3	1	**0**	0
Rancho San Diego *(Martin)*		2	3	**0**	0
Saber Springs Parkway		1	3	**0**	0
Steele Canyon *(Martin)*		1	5	**0**	0
Trail 62		1	1	**0**	0
Totals:		61 Routes	136 Visits	**183 Total Hc**	65 Max Hc

We made a total of 136 surveys in the six week flight season (Table 8), most of which occurred in the four weeks between May 31st and June 25th. We counted a total of 183 Hermes copper adult observations distributed across 13 occupied sites. Five of these sites were areas where Hermes copper had not formally been reported (Hermes were reported in several parts of McGinty Mountain previously, but we found a new section with Hermes copper and count it as a new sighting since the area is so large). Of the 13 sites with Hermes only three had single day max counts greater than 5 individuals (Skyline Truck Trail, Sycuan Peak, and McGinty Mountain). The same three sites were the only ones that had total season counts (Pollard Total) of ten (Table 8, Figure 5).

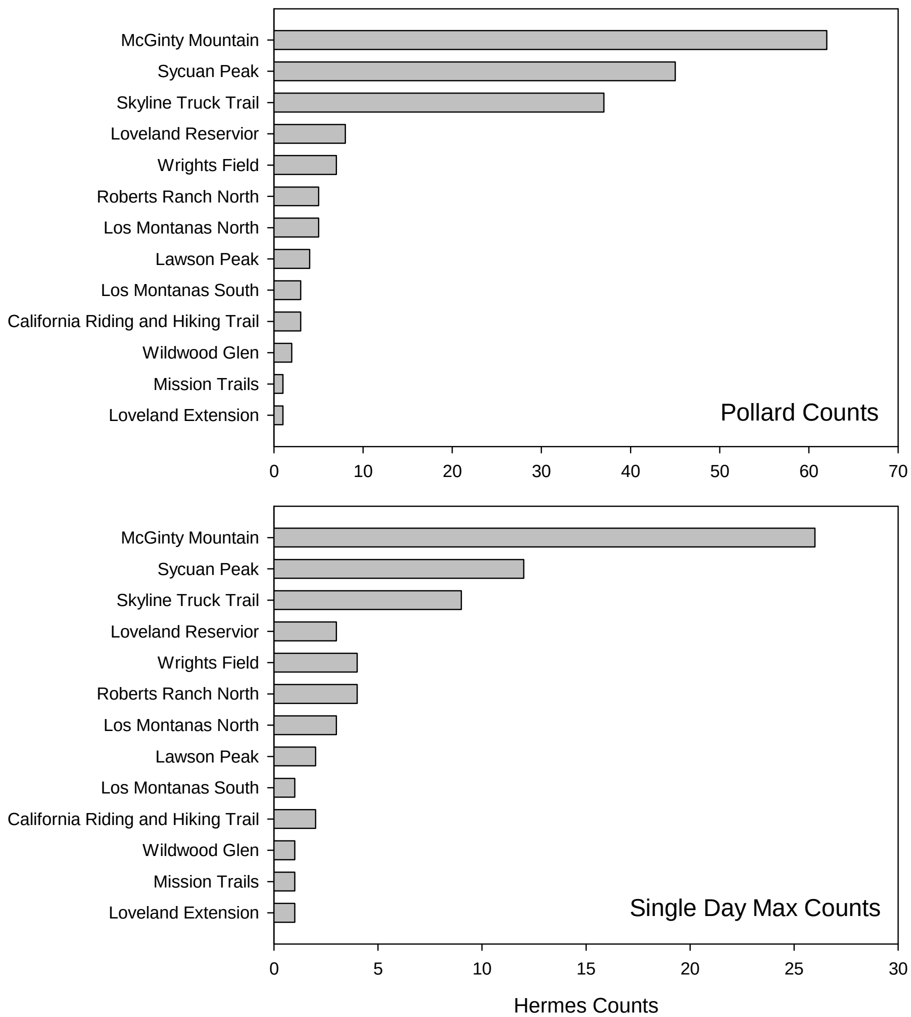
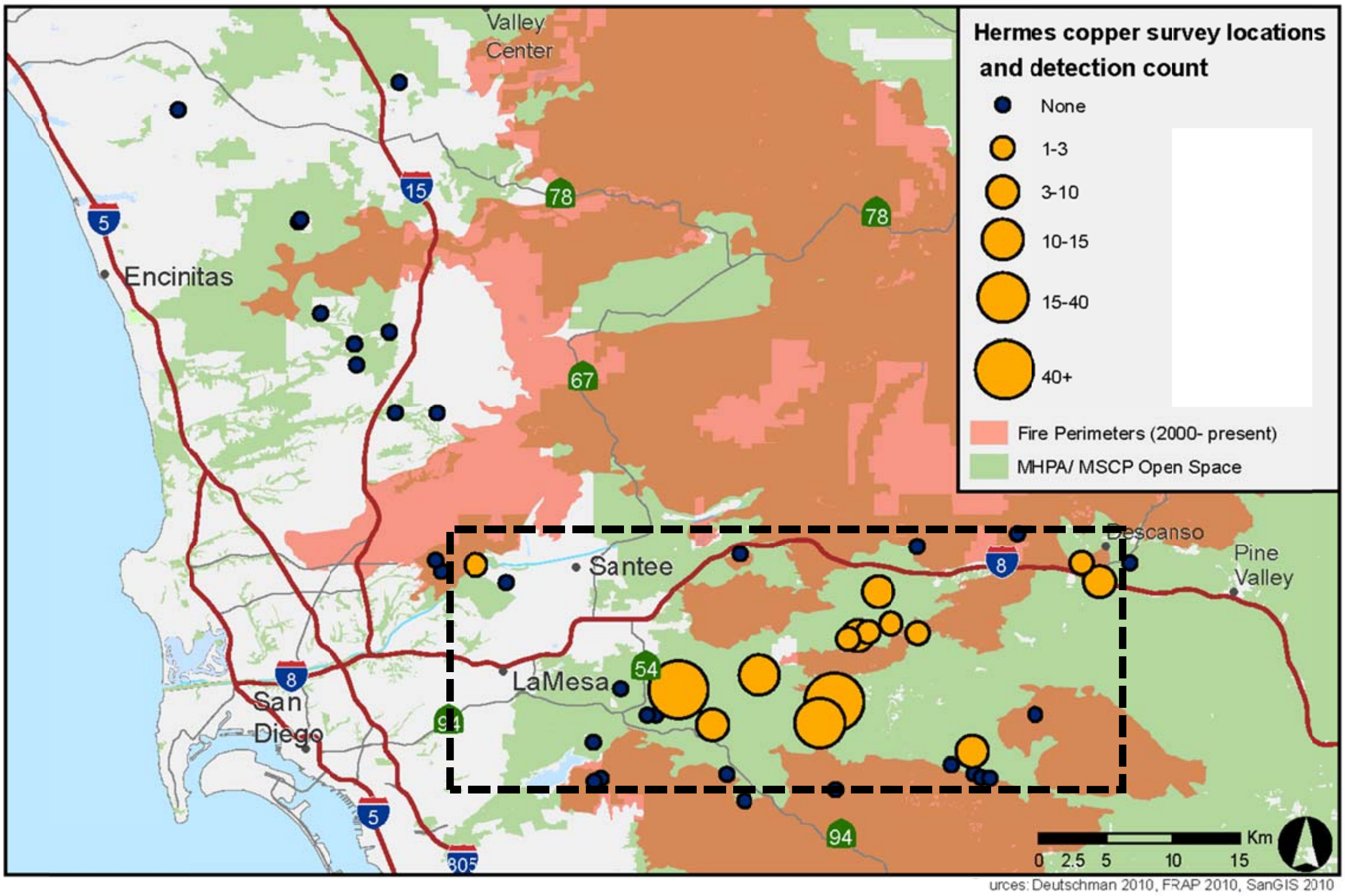


Figure 5: Pollard and max counts for all sites with Hermes copper butterflies. Pollard counts are the sum of all individuals recorded during the flight season.

Although we looked as far north and west as Vista, our northern and western most Hermes copper observation was made at Kwaay Paay Peak in Mission Trails Regional Park (Map 5). We looked as far east as Guatay Mountain, and made our eastern most observation nearby at Robert's Ranch in the Descanso area. Although we looked as far south as Rancho Jamul, our southernmost observation was made at Lawson Peak. In total, Hermes copper occupied an area of approximately 1,000 km^2 (see Map 5) which represents approximately 9% of the land area of San Diego county (based on land area estimate of 10,878 km^2 from US Census Bureau).



Map 5: Detections of Hermes copper butterflies on conserved lands, 2010. Black circles mark sites with no detections. Orange circles represent sites with Hermes copper. Circle size is proportional to the total number of Hermes copper butterflies recorded (Pollard Index). The dashed box is a 50km by 20km (area = 1000 km^2) rectangle that encloses all the individuals that we detected.

Task E – *Synthesis and Comprehensive Analysis*

As mentioned in the previous section the flight season did not start until May 29th, about 2 weeks later than our anticipated Hermes copper emergence date. We began sampling when the first Hermes emerged at the Skyline Truck Trail site. Most sites did not have butterflies that week, however the season picked up rapidly. By the week of June 7 (Mon) through Jun 13 (Sun), we detected Hermes copper at a maximum of 12 sites occupied (Figure 6, gray circles). We also observed peak densities the same week (Figure 6, white triangles). The season tailed off gradually from there.

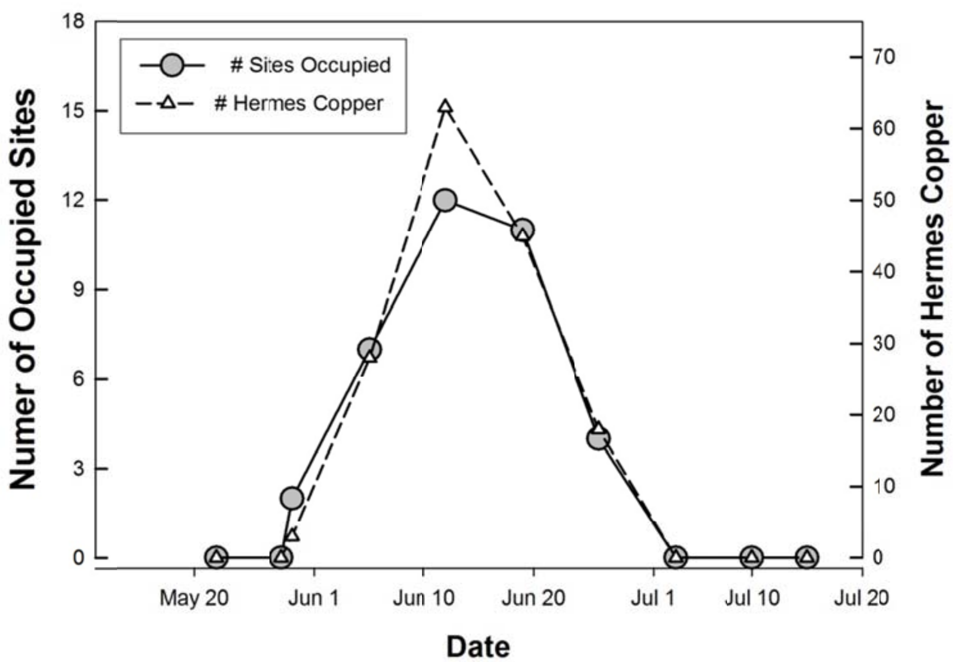


Figure 6: Hermes copper distribution and population size through the flight season. Data are summarized weekly.

The pattern of a sharp increase in adult numbers during the beginning of the season and a gradual decline after the season peak is fairly typical for butterfly populations; however, this trend was more variable when considering each site individually (Figure 7). The sites with the smallest daily counts tended to have the shortest flight season, indicating that only a few individuals ever emerged this year (as opposed to many emerging with prolonged emergence period).

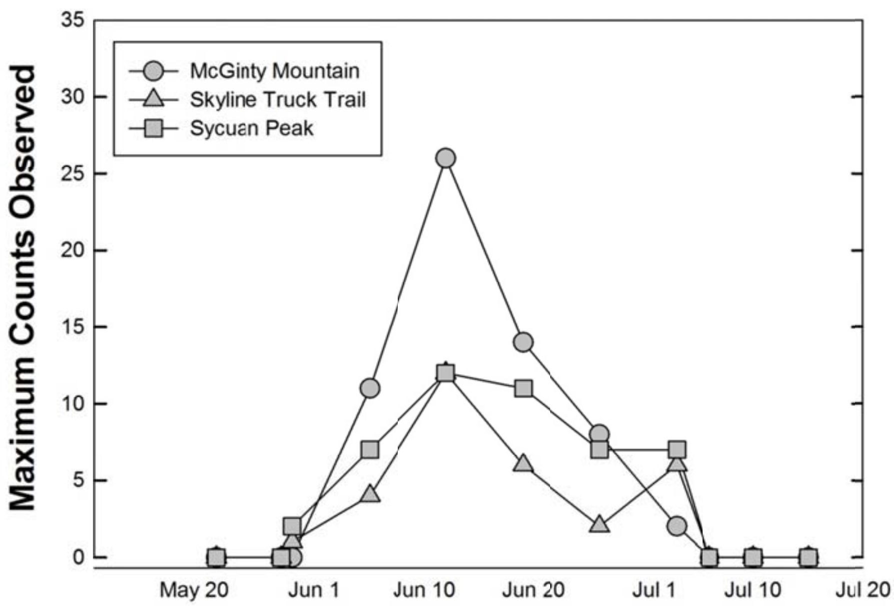


Figure 7: Hermes copper distribution and population size at McGinty Mountain, Skyline Truck Trail, and Sycuan Peak. Data are summarized weekly.

Low count numbers also make analyzing the relationship of Hermes copper to environmental factors difficult. Marschalek showed that Hermes in captivity have a threshold for becoming active around 22°C (72°F) and field observations indicate adults tend to seek shade in the vegetation at high temperatures. We made the same observation regarding the minimum temperature threshold for activity, with only 1 observation of Hermes made below 22°C. We did not, however, observe a correlation between temperature and the number of Hermes copper observed, as long as we surveyed at greater than 22°C. The upper threshold for Hermes activity it is not clear as most of our observations were made below 31°C (87°F) degrees. It was an extremely mild year and we did not have any opportunities to make observations at higher temperatures.

In addition to being cool, 2010 was the first above average rainfall year since 2005 (as measured at the Otay Lakes weather station which is close to the center of the historical Hermes range) (Figure 8). The years of 2007, 2008 and 2009 each had between 2 and 4 inches less rain than average. Although periods of drought are frequent in San Diego County, the window between 2006 and 2009 represents the longest dry period over the last 12 years (since 1999). This dry period could be one factor effecting Hermes copper populations in 2010. Other research has clearly demonstrated the importance of precipitation to adult butterfly numbers (Pollard 1988, Roy et al. 2001).

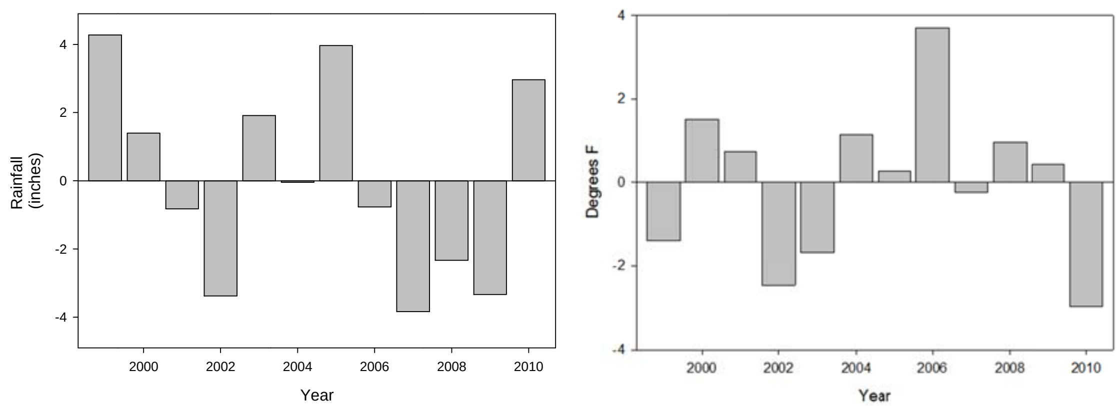


Figure 8: Deviations from average rainfall (left) and maximum temperature (right) at Otay Lakes, San Diego, CA. Rainfall anomalies are based on January through April totals. Temperature anomalies are based on April through June values.

Conclusions

We documented Hermes copper at 13 of 35 sites that were identified as potential high-quality habitat. Most of these occupied sites had less than 10 total butterflies observed over the entire flight season. In total, we counted only 186 individuals over the course of 136 site visits county-wide. This is an alarmingly small number, especially considering some of the 186 individuals counted were likely observations of the same individual on subsequent visits. In addition, more than 85% of all counts were concentrated at three sites located close together. As a result, the majority of individuals could be lost during a single catastrophic event such as the wildfires observed in 2003 or 2007.

To the best of our knowledge, Hermes copper were detected at an additional 4 sites surveyed by other individuals.(Table 2). In addition, we did not repeat surveys along the Sunrise Powerlink area since they were surveyed in 2009 and no substantial changes have been made to the habitat. Even given these additional sites, it is clear that the range of Hermes copper is substantially smaller than the range based on historical records.

Table 9: Other known sites containing Hermes copper in 2010.

Site	Observer
South of Skyline Truck Trail	Gretchen Cummings (Cummings and Associates)
Protrero	Michael Klein
Bell Bluff	Michael Klein
Steele Canyon	Jocelyn Robbins

The density of Hermes copper is harder to estimate because of their patchy distribution, low numbers, and the different behavior of males (territorial) versus females (longer distances). Despite the uncertainty, our counts seem low compared to previous work. For example, our highest daily count was 26 individuals at McGinty mountain. In 2003, Dan Marschalek observed much higher maximum counts at two of his four sites (Crestridge and Anderson Road, Marschalek and Deutschman 2008). Moreover, he observed counts above 35 on four separate surveys at both sites. Marschalek and Klein (2010) published data from four years of data at Rancho Jamul Ecological Reserve. They demonstrated that maximum counts can vary by an order of magnitude from year to year (Table 2 in Marschalek and Klein). Given that natural variability, it is difficult to draw any conclusions about density from our single-year sample.

Although the spring of 2010 was unusually mild, we do not have adequate data to conclude that low temperatures or the dry period preceding 2010 are responsible for the low densities of Hermes copper observed this year. We cannot with confidence predict that warmer temperatures would lead to more individuals at the occupied sites. Although we surveyed sites exhaustively throughout the flight season, we cannot be certain that unoccupied sites do not contain Hermes copper. It is possible that individuals were not detected or that eggs/larvae have undergone diapause (and thus did not fly).

Despite the comprehensive design of our surveys, we are not able to reach a firm conclusion on the status of Hermes copper. Hermes copper population sizes can vary year to year (Marschalek and Klein 2010). We recommend continuing surveys next year at all 35 sites before concluding that Hermes copper is absent at any of those sites.

Although the number of unburned sites with potential habitat is small, we have identified a number of small sites to do additional redberry reconnaissance searches including:

- Sandia Creek (a different location than searched this year)
- Daley Ranch (different location than searched this year)
- Dixon Lake
- Lake Wholford
- Agua Tibia Mountain/ Arroyo Seco (at appropriate locations)
- Dictionary Hill

If redberry is found at any of these sites they should be added to next year's surveys.

Finally, it is important to understand Hermes dispersal ability as it is a critical in understanding recolonization of suitable habitat following fires. These questions may be answered by using molecular techniques. In 2011 we will continue to process specimens and continue the genetic analysis started in 2010.

The results from our 2010 field surveys suggest that Hermes copper populations are limited to a small portion of San Diego County. This area is substantially smaller than the historic range of the population. In addition, the number of individual Hermes copper butterflies counted was quite small. Although numbers may rebound in subsequent years, there is ample cause for concern. Further monitoring of this species is necessary to gauge the severity of risk facing the species. In the meantime, it is prudent to assume that the populations are small in size and restricted in distribution and thus at risk of extinction.

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Appendix 1: Redberry Search Information

Site Name	Redberry	Lat	Long
Alpine-- Wrights Field†	Multiple Dense Patches	32.826759°	-116.766878°
Barrett Lake†	Multiple Dense and Moderate Patches	32.704020°	-116.719120°
Bette Bendixen Mini-Park	Single Moderate Patch	32.944080°	-117.068780°
Black Mountain North	Single Sparse Patch	32.997890°	-117.099760°
Black Mountain South†	Single Moderate Patch	32.977280°	-117.116320°
Black Mountain West	Multipel Moderate Patches	32.988130°	-117.122200°
Buena Vista Park	Single Individual	33.153850°	-117.246520°
California Riding and Hiking Trail	Multiple Moderate and Sparse Patches	32.799850°	-116.762260°
Cowles Mountain	Multiple Dense Patches	32.826909°	-117.020448°
Crestridge Ecological Reserve*	Multiple Moderate Patches-- Burned	32.823450°	-116.864128°
Daley Ranch	None	33.169776°	-117.052539°
Damon Lane County Park	Single Moderate Patch	32.756530°	-116.943540°
Dehesa Rd. (granite hills)/SDNWR	None	32.768398°	-116.886365°
Del Dios Highlands Preserve	Not Searched-- Discovered it was burned	--	--
Descanso-- Boulder Creek Road†	Scattered Individuals-- Burned	32.882600°	-116.646100°
Descanso-- Viejas Blvd	None	32.856800°	-116.607104°
Descanso-- Wildwood Glen*	Multiple Moderate Patches	32.841182°	-116.631731°
El Capitan	None / Burned	32.895418°	-116.814241°
El Monte County Park	Single Individual	32.891420°	-116.846070°
Elfin Forest / Harmony Grove Rd.	Multiple Dense and Moderate Patches	33.074880°	-117.159320°
Flynn Spring County Park	Two Moderately Dense Patches	32.846430°	-116.861400°
Guatay Mountain East	Scattered Individuals	32.832237°	-116.570214°
Guatay Mountain West	Multiple Dense and Moderate Patches	32.836420°	-116.596070°
Hell Hole Canyon Preserve	None / Burned	33.216940°	-116.931853°
Hollenbeck Canyon*	Multiple Moderate Patches-- Burned	32.695022°	-116.811726°
Jesmond Dene Park	Single Dense Patch	33.168110°	-117.094730°
La Jolla Canyon	Scattered Individuals	33.003360°	-117.152340°
Lake Jennings Park	Single Individual	32.881100°	-116.840600°
Lake Murray	Scattered Individuals	32.788230°	-117.048290°
Lake San Marcos/Discovery Lake	None	33.123805°	-117.178373°
Lake Wholford Road	Single Individual	33.222720°	-116.974050°
Lakeside Linkage	None	32.841131°	-116.913458°
Lawson Peak*	Multiple Dense and Moderate Patches	32.714540°	-116.705660°
Los Montanas North†	Multiple Dense Patches	32.732410°	-116.894360°
Los Montanas South†	Single Dense Patch	32.727810°	-116.898560°
Los Penasquitos Canyon Preserve	None	32.934189°	-117.147096°
Loveland Extension	Multiple Dense and Moderate Patches	32.790270°	-116.742910°

Site Name	Redberry	Lat	Long
Loveland Reservoir	Multiple Moderate and Sparse Patches	32.797370°	-116.772220°
Lyons Valley (Sunrise powerlink)†	Not Searched-- Others doing surveys	--	--
Marron Valley	Scattered Individuals-- Burned	32.572258°	-116.754723°
Meadowbrook-- Meadowbrook Lane*	Multiple Moderate Patches	32.963152°	-117.069400°
Meadowbrook--Shoal Creek Drive	Single Moderate Patch	32.963511°	-117.080455°
Mission Trails -- East†	Scattered Individuals	32.840306°	-117.043245°
Mission Trails-- Kwayy Pai Peak	Single Moderate Patch	32.833799°	-117.040599°
Mission Trails-- West	Multiple Moderate Patches-- Burned	32.836027°	-117.063926°
Oak Oasis	None	32.914926°	-116.894975°
Oak Riparian Park	None	33.176381°	-117.269747°
Otay Mesa	Single Individual	32.549220°	-116.998050°
Poser Mountain	Two Moderately Dense Patches-- Burned	32.864910°	-116.661660°
Rancho Jamul*	Multiple Dense and Moderate Patches	32.674148°	-116.862625°
Roberts Ranch North†	Multiple Moderate and Sparse Patches	32.825979°	-116.615765°
Saber Spring's Parkway	Single Dense Patch	32.943670°	-117.095830°
Santa Margarita Preserve	None	33.406345°	-117.261521°
SDNWR-- McGinty Mtn East†	Multiple Dense and Moderate Patches	32.755829°	-116.855599°
SDNWR-- McGinty Mtn South†	Multiple Dense and Moderate Patches	32.742138°	-116.864513°
SDNWR-- McGinty Mtn West and North†	Multiple Dense Patches	32.763977°	-116.874311°
SDNWR-- Sloane Canyon Road	Multiple Moderate and Sparse Patches	32.763420°	-116.844800°
SDNWR-- Steele Canyon	Multiple Dense Patches	32.737260°	-116.925710°
Skyline Truck Trail*	Single Moderate Patch	32.732088°	-116.806096°
Swartz Canyon County Park	Not Searched-- Discovered it was burned	--	--
Sycamore Canyon County Park*	Not Searched-- Discovered it was burned	--	--
Sycuan Peak	Multiple Dense and Moderate Patches	32.747260°	-116.799790°
Trail 62	Scattered Individuals	32.737620°	-116.663420°
Turner Lake	Single Individual	33.226840°	-117.081400°
Viejas Mountain/ Anderson Truck Trail*	Single Moderate Patch-- Burned	32.854930°	-116.741620°
Wilderness Gardens Park	Single Individual	33.347180°	-117.025620°

Appendix 2: Hermes Copper Locations

Case	Date	Time	Site	Route	N	W	Altitude (')
1	5/29/2010	1:04 AM	SYP	1	32.74819	-116.80003	656
3	5/29/2010	2:21 AM	SYP	1	32.75016	-116.80049	709
2	5/29/2010	11:59 AM	STT	1	32.73205	-116.80726	621
4	5/30/2010	12:52 PM	STT	1	32.73206	-116.80727	629
5	5/31/2010	10:05 AM	STT	1	32.73207	-116.80722	629
6	6/2/2010	1:02 AM	SYP	1	32.75036	-116.80041	715
7	6/2/2010	1:44 AM	LMS	1	32.72767	-116.89955	611
8	6/2/2010	11:29 AM	SYP	1	32.74731	-116.79979	625
9	6/2/2010	11:37 AM	SYP	1	32.74768	-116.79983	646
10	6/2/2010	11:43 AM	SYP	1	32.74819	-116.80000	659
11	6/2/2010	11:48 AM	SYP	1	32.74869	-116.80034	670
12	6/2/2010	11:53 AM	SYP	1	32.74884	-116.80013	674
13	6/2/2010	11:59 AM	SYP	1	32.74977	-116.80045	698
14	6/2/2010	12:14 PM	LMN	1	32.73778	-116.89577	577
15	6/3/2010	10:17 AM	LLR	1	32.79008	-116.77864	1447
16	6/3/2010	10:25 AM	STT	1	32.73200	-116.80725	627
17	6/3/2010	10:33 AM	STT	1	32.73205	-116.80652	620
18	6/4/2010	1:00 AM	MGM	2	32.76451	-116.87407	858
19	6/4/2010	1:24 AM	MGM	2	32.76836	-116.87137	932
20	6/4/2010	1:31 AM	MGM	2	32.76884	-116.87017	921
21	6/4/2010	1:35 AM	MGM	2	32.76842	-116.86935	949
22	6/4/2010	1:55 AM	MGM	2	32.76630	-116.86186	1089
23	6/4/2010	11:15 AM	MGM	1	32.75740	-116.85440	1400
24	6/4/2010	11:23 AM	MGM	2	32.75945	-116.87831	1053
25	6/4/2010	11:33 AM	MGM	1	32.75550	-116.85605	1547
26	6/4/2010	11:47 AM	MGM	1	32.75451	-116.85665	1613
27	6/4/2010	12:38 PM	MGM	2	32.76405	-116.87428	837
28	6/4/2010	12:57 PM	MGM	2	32.76449	-116.87441	841
29	6/6/2010	10:17 AM	WF	1	32.82124	-116.76919	1949
30	6/6/2010	10:37 AM	WF	1	32.82180	-116.77024	1870
31	6/6/2010	11:08 AM	WF	1	32.82169	-116.77068	1880
34	6/7/2010	10:54 AM	STT	1	32.73208	-116.80718	616
35	6/7/2010	10:57 AM	STT	1	32.73211	-116.80641	612
36	6/7/2010	11:00 AM	STT	1	32.73181	-116.80587	615
37	6/7/2010	11:06 AM	STT	1	32.73198	-116.80600	621
38	6/7/2010	11:10 AM	STT	1	32.73216	-116.80786	634
39	6/7/2010	12:01 PM	MT	4	32.83671	-117.03986	483

Case	Date	Time	Site	Route	N	W	Altitude (')
32	6/7/2010	.	CRH	1	32.79976	-116.76218	1454
33	6/7/2010	.	LLE	1	32.79115	-116.74407	1364
40	6/8/2010	1:01 AM	SYP	1	32.74795	-116.79986	650
41	6/8/2010	1:07 AM	SYP	1	32.74842	-116.80003	663
42	6/8/2010	1:10 AM	SYP	1	32.74861	-116.80073	669
43	6/8/2010	1:10 AM	SYP	1	32.74861	-116.80073	668
44	6/8/2010	1:12 AM	SYP	1	32.74871	-116.80033	669
45	6/8/2010	1:17 AM	SYP	1	32.74918	-116.80024	681
46	6/8/2010	1:20 AM	SYP	1	32.74961	-116.80039	689
47	6/8/2010	1:36 AM	SYP	1	32.74983	-116.80045	699
48	6/8/2010	1:46 AM	SYP		32.74823	-116.79998	657
49	6/8/2010	1:51 AM	SYP		32.74763	-116.79959	639
50	6/8/2010	10:23 AM	LAW	1	32.71715	-116.71244	2643
51	6/8/2010	10:39 AM	WWG	1	32.84191	-116.63976	1009
52	6/8/2010	10:40 AM	LAW		32.71996	-116.71510	2848
53	6/8/2010	12:52 PM	SYP	1	32.74711	-116.79998	622
54	6/8/2010	12:56 PM	SYP	1	32.74744	-116.79948	625
57	6/9/2010	1:09 AM	MGM	2	32.76767	-116.86478	318
58	6/9/2010	1:15 AM	MGM	2	32.76620	-116.86176	330
59	6/9/2010	1:20 AM	MGM	2	32.76544	-116.85954	333
60	6/9/2010	1:23 AM	MGM	2	32.76468	-116.85906	336
61	6/9/2010	1:29 AM	MGM	2	32.76322	-116.85771	332
62	6/9/2010	1:55 AM	MGM	2	32.75930	-116.85086	350
63	6/9/2010	11:04 AM	MGM	1	32.75766	-116.85431	1406
64	6/9/2010	11:05 AM	MGM	1	32.75742	-116.85438	1412
65	6/9/2010	11:24 AM	MGM	1	32.75665	-116.85536	1491
66	6/9/2010	11:32 AM	MGM	1	32.75647	-116.85544	1499
67	6/9/2010	11:35 AM	MGM	1	32.75610	-116.85560	1509
68	6/9/2010	11:48 AM	MGM	1	32.75454	-116.85664	1620
69	6/9/2010	12:11 PM	MGM	2	32.76382	-116.87408	259
70	6/9/2010	12:16 PM	MGM	2	32.76408	-116.87439	254
71	6/9/2010	12:16 PM	MGM	2	32.76407	-116.87435	254
72	6/9/2010	12:16 PM	MGM	2	32.76406	-116.87435	254
73	6/9/2010	12:19 PM	MGM	2	32.76447	-116.87436	255
74	6/9/2010	12:21 PM	MGM	2	32.76446	-116.87423	258
75	6/9/2010	12:23 PM	MGM	1	32.75262	-116.85793	1819
76	6/9/2010	12:24 PM	MGM	2	32.76450	-116.87403	257
77	6/9/2010	12:30 PM	MGM	2	32.76551	-116.87391	263
78	6/9/2010	12:40 PM	MGM	2	32.76821	-116.87177	284
79	6/9/2010	12:49 PM	MGM	2	32.76886	-116.87007	278

Case	Date	Time	Site	Route	N	W	Altitude (')
80	6/9/2010	12:52 PM	MGM	2	32.76839	-116.86932	282
81	6/9/2010	12:54 PM	MGM	2	32.76804	-116.86884	291
82	6/9/2010	12:58 PM	MGM	2	32.76824	-116.86792	294
55	6/9/2010	.	LMN	1	32.73782	-116.89575	581
56	6/9/2010	.	LMS	1	32.72697	-116.89980	627
83	6/10/2010	1:09 AM	LLR	3	32.79019	-116.78267	1435
84	6/10/2010	1:17 AM	LLR	3	32.79097	-116.78304	1426
85	6/10/2010	12:12 PM	LLR	1	32.79153	-116.77704	1510
86	6/11/2010	1:18 AM	STT	1	32.73206	-116.80726	623
87	6/11/2010	1:22 AM	STT	1	32.73211	-116.80659	626
88	6/11/2010	1:24 AM	STT	1	32.73212	-116.80641	614
89	6/11/2010	1:28 AM	STT	1	32.73211	-116.80626	613
90	6/11/2010	1:34 AM	STT	1	32.73204	-116.80607	612
91	6/11/2010	1:35 AM	STT	1	32.73188	-116.80615	618
92	6/11/2010	1:37 AM	STT	1	32.73178	-116.80589	613
93	6/13/2010	10:37 AM	WF	1	32.82133	-116.76879	1870
94	6/13/2010	11:39 AM	WF	1	32.82250	-116.77064	1824
95	6/14/2010	10:05 AM	CRH	1	32.80033	-116.76336	1532
97	6/15/2010	1:54 AM	STT	1	32.73217	-116.80785	634
98	6/15/2010	1:56 AM	STT	1	32.73204	-116.80726	627
99	6/15/2010	1:57 AM	STT	1	32.73203	-116.80723	627
101	6/15/2010	2:01 AM	STT	1	32.73206	-116.80666	625
102	6/15/2010	2:03 AM	STT	1	32.73211	-116.80610	616
103	6/15/2010	2:04 AM	STT	1	32.73175	-116.80582	613
104	6/15/2010	9:37 AM	LMN	1	32.73145	-116.88017	815
105	6/15/2010	9:56 AM	LMN	1	32.73124	-116.88149	865
100	6/15/2010	12:25 PM	LMS	1	32.72696	-116.89983	650
96	6/15/2010	.	LMN	2	32.73769	-116.89544	605
106	6/16/2010	1:15 AM	RRN	1	32.82701	-116.61555	1073
107	6/16/2010	1:18 AM	RRN	1	32.82757	-116.61524	1082
108	6/16/2010	1:18 AM	RRN	1	32.82757	-116.61524	1082
109	6/16/2010	1:25 AM	RRN	1	32.82785	-116.61445	1087
111	6/16/2010	9:50 AM	LAW	1	32.71547	-116.70668	695
112	6/16/2010	9:50 AM	LAW	1	32.71548	-116.70668	695
110	6/16/2010	11:54 AM	WWG	1	32.84224	-116.64175	1015
113	6/17/2010	.	LLR	3	32.79136	-116.78326	1447
114	6/17/2010	.	SYP	1	32.74761	-116.79953	2087
115	6/17/2010	.	SYP	1	32.74817	-116.79995	2145
116	6/17/2010	.	SYP	1	32.74855	-116.80070	2181
117	6/17/2010	.	SYP	1	32.74958	-116.80029	2259

Case	Date	Time	Site	Route	N	W	Altitude (')
118	6/17/2010	.	SYP	1	32.75003	-116.80046	2304
119	6/17/2010	.	SYP	1	32.75299	-116.80385	2654
120	6/17/2010	.	SYP	1	32.75325	-116.80475	2717
121	6/17/2010	.	SYP	1	32.75283	-116.80229	2598
122	6/17/2010	.	SYP	1	32.75200	-116.80150	2515
123	6/17/2010	.	SYP	1	32.74867	-116.80043	2207
124	6/17/2010	.	SYP	1	32.74868	-116.80035	2209
126	6/18/2010	1:01 AM	MGM	2	32.76545	-116.86043	1115
127	6/18/2010	1:02 AM	MGM	2	32.76545	-116.86038	1115
128	6/18/2010	1:07 AM	MGM	2	32.76543	-116.85953	1118
129	6/18/2010	1:12 AM	MGM	2	32.76417	-116.85816	1131
130	6/18/2010	1:17 AM	MGM	2	32.76319	-116.85770	1123
131	6/18/2010	11:49 AM	MGM	2	32.76408	-116.87438	843
132	6/18/2010	11:55 AM	MGM	2	32.76566	-116.87378	884
133	6/18/2010	12:15 PM	MGM	2	32.76826	-116.87170	932
134	6/18/2010	12:17 PM	MGM	2	32.76832	-116.87141	929
135	6/18/2010	12:21 PM	MGM	2	32.76884	-116.87017	920
136	6/18/2010	12:27 PM	MGM	2	32.76843	-116.86932	941
137	6/18/2010	12:45 PM	MGM	2	32.76785	-116.86611	1023
138	6/18/2010	12:50 PM	MGM	2	32.76764	-116.86467	1054
125	6/18/2010	.	MGM	1	32.747238°	-116.86205	.
139	6/20/2010	1:07 AM	WF	1	32.82197	-116.76934	1847
144	6/22/2010	9:30 AM	SYP	1	32.74688	-116.79954	1991
145	6/22/2010	9:45 AM	SYP	1	32.74850	-116.80067	2154
146	6/22/2010	9:48 AM	SYP	1	32.74871	-116.80037	2166
140	6/22/2010	10:36 AM	SYP	1	32.75201	-116.80154	2520
141	6/22/2010	10:47 AM	SYP	1	32.75280	-116.80235	2581
142	6/22/2010	10:55 AM	SYP	1	32.75300	-116.80387	2656
143	6/22/2010	11:00 AM	SYP	1	32.75325	-116.80475	2705
149	6/23/2010	1:27 AM	MGM	2	32.76541	-116.85949	1122
150	6/23/2010	1:34 AM	MGM	2	32.76410	-116.85814	1110
151	6/23/2010	12:02 PM	MGM	2	32.76404	-116.87432	824
152	6/23/2010	12:31 PM	MGM	2	32.76564	-116.87375	896
153	6/23/2010	12:44 PM	MGM	2	32.76820	-116.87166	947
154	6/23/2010	12:48 PM	MGM	2	32.76837	-116.87129	937
147	6/23/2010	.	MGM	1	32.75568	-116.85603	1413
148	6/23/2010	.	MGM	1	32.73717	-116.86800	1417
155	6/24/2010	.	LLR	3	32.79145	-116.78330	1440
156	6/24/2010	.	LLR	4	32.78962	-116.79012	1424
157	6/25/2010	12:00 PM	RRN	1	32.82759	-116.61500	3563

Case	Date	Time	Site	Route	N	W	Altitude (')
158	6/28/2010	1:05 AM	STT	1	32.73217	-116.80786	2094
159	6/28/2010	1:10 AM	STT	1	32.73203	-116.80721	2054
160	6/28/2010	1:16 AM	STT	1	32.73208	-116.80653	2034
161	6/28/2010	1:24 AM	STT	1	32.73169	-116.80589	2087
162	6/28/2010	11:09 AM	SYP	1	32.75326	-116.80477	2714
163	6/28/2010	11:20 AM	SYP	1	32.75286	-116.80226	2591
164	6/28/2010	11:28 AM	SYP	1	32.75199	-116.80142	2500
165	6/28/2010	11:34 AM	SYP	1	32.75038	-116.80008	2346
166	6/28/2010	11:57 AM	SYP	1	32.74874	-116.80035	2207
167	6/28/2010	12:26 PM	SYP	1	32.74690	-116.79950	2020
168	6/29/2010	.	MGM	2	32.44350	-116.52120	.
169	6/29/2010		MGM	2	32.46400	-116.52900	.
170	6/30/2010	11:51 AM	LLR	4	32.78453	-116.79211	1457
171	7/2/2010	10:41 AM	SYP	1	32.74869	-116.80034	2203
172	7/2/2010	12:31 PM	STT	1	32.73182	-116.80812	2289
173	7/2/2010	12:39 PM	STT	1	32.73210	-116.80614	2018
174	7/2/2010	12:41 PM	STT	1	32.73197	-116.80595	2029
175	7/2/2010	12:44 PM	STT	1	32.73176	-116.80587	2019